

Reproductive biology of lotak, *Cyprinion macrostomum* Heckel, 1843 (Pisces: Cyprinidae), from the Tigris River drainage

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Abstract

Some reproduction aspects of the "doctor fish", *Cyprinion macrostomum*, were examined for the first time from the Tigris River drainage. A total of 316 specimens were caught from Gamasiab River in western Iran, at monthly intervals throughout the year from January to December 2013. Age, sex ratio, fecundity, oocytes diameter, gonado-somatic (GSI), modified gonado-somatic (MGSI) and Dobriyal (DI) indices were estimated. Regression analyses were used to find relations between fecundity and fish size (length and weight), gonad weight, and age. Sex ratio differed significantly from unity, and the percentage of males was greater than that of females. The mature females and males were longer than 108 mm and 94 mm in total length (+2 and +1 in age, respectively). The GSI ranged between 0.09-2.94% and 1.70-15.53% for males and females, respectively. Gonad indices showed spawning took place from May to August, when the water temperature was 16 to 24 °C. The average diameter of the oocytes ranged from 0.2 mm to 1.7 mm, in the spawning season. The averages of absolute and relative fecundity (relative to body weight) were 3642.51 eggs (SD ±1219.92) and of 55.04 (SD ±14.12) per gram body weight, respectively. The absolute fecundity was significantly related to body weight and ovary weight.

Keywords: Fecundity, Maturity, Oocytes diameter, Gonado-somatic, Iran

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Introduction

The genus *Cyprinion* Heckel, 1843 a member of the family Cyprinidae comprises five species in Iran, including *C. kais*, *C. macrostomum*, *C. milesi*, *C. tenuiradius* and *C. watsoni* (Banarescu and Herzig-Straschil, 1995; Abdoli, 2000; Coad, 2016). In Iran, this genus is found in the Tigris River drainage including the Hawr Al Azim, Khersan, Jarrahi, Marun and Gamasiab Rivers (Berg, 1949; Abdoli, 2000), Mashkid, Makran and Jazmurian basins (Coad, 2016), and the northern Persian Gulf basin in the Shapur, Dalaki and Helleh rivers (Bibak *et al.*, 2013; Jouladeh-Roudbar *et al.*, 2015). The *C. macrostomum* Heckel, 1843 (Fig. 1) is widely distributed in the Tigris-Euphrates drainages, in Iran, Iraq, Syria and Turkey. Ündar *et al.* (1990) identify *C. macrostomum* and *Garra rufa* as the "doctor fish" of the Kangal hot spring in Turkey. High water temperatures reduce the amount of plankton available as fish food and the fish nibble away infected skin of humans who bathe in these waters. The *C. macrostomum* is known as "striker" (and *G. rufa* as "licker") from its behavior in the spa pools (Coad, 2016). Also, Al-Mehdi and Khan (1984) reported this species to be important in riverine and culture fisheries in northern Iraq. Although there is a wide distribution of *C.*

macrostomum in the Tigris-Euphrates catchment and its importance as a commercial species is known, there is no available details on its biological aspects. Reproduction represents one of the most important aspects of the different species biology and the maintenance of viable populations depending on its success (Suzuki and Agostinho, 1997). Knowledge of gonadal development and the spawning season of a species allow subsequent studies on spawning frequency of its population, which is important for its management (Chakraborty *et al.*, 2007). Study of sex-ratio, length at first sexual maturity, cycle of maturation and spawning periodicity are important aspects of studies on reproduction biology of any fish species (Reddy, 1979; Vazzoler, 1996). There is some information about the morphological and genetic diversity of *Cyprinion* fishes (Daştan *et al.*, 2012; Nasri *et al.*, 2013) but little is known of its ecological and reproductive features. Therefore, the present study aimed to providing data on the reproductive biology aspects, including age, sex ratio, gonado-somatic index, fecundity, oocytes diameter, and spawning season which are necessary for commercial harvesting, artificial propagation and conservation programs of this fish.



Figure 1: The "doctor fish" *Cyprinion macrostomum*, 142 mm SL, from the Gamasiab River.

Materials and methods

A total of 316 specimens were caught by electrofishing (energy level: 200–300 V; distance between electrodes: 10–50 meters; area covered each time: 100 meters; water depth covered: 40–120 cm; substrate of the fishing areas: with diverse structures including pools, riffles, gravel bed, sandy shore, etc.) from Gamasiab River in the Tigris River drainage (34°22'249"N, 047°54'729"E), in Kermanshah Province (Fig. 2).

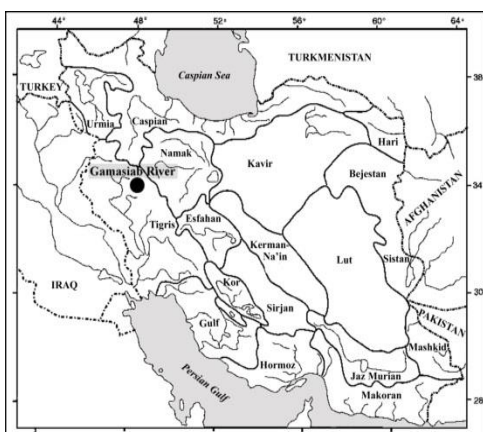


Figure 2: The map of Iranian basins, showing the Tigris River drainage and Gamasiab River.

The Gamasiab River is one of the most important rivers in terms of fish diversity in the region (Biukani *et al.*, 2013), located in Hamedan and

Kermanshah Provinces, in western Iran. Sampling was done at monthly intervals throughout the year at the same sampling site, from January 2013 to December 2013. The sampling site was selected based on presence and density of the fish. After anesthesia, specimens were fixed in 10% formaldehyde.

Total length (TL), standard length (SL) and fork length (FL) of each specimen was measured to the nearest 1.0 mm by using a dial caliper. Total body weight (W_b) was measured by a digital balance with 0.01 g accuracy. After dissection, sex and maturity were determined by macroscopic examination of the gonads. The ratio of males to females was tested with the chi-square test (Zar, 1999). To measure age, scales were removed from the left side of the fish just between the dorsal fin and above the lateral line and cleaned with 5% NaOH for age determination. Age was determined with a microscope (model M6C–10) after the scales were placed between slides (Mousavi-Sabet *et al.*, 2011; 2012a; 2012b; Mousavi-Sabet, 2012;). The reproductive period was determined by using three gonad

indices: gonado-somatic index (GSI), modified gonado-somatic index (MGSI) and Dobriyal index (D.I.) (Mousavi-Sabet *et al.*, 2011; 2012a; 2012b). In order to determine the $GSI = (W_g \times W_b^{-1}) \times 100$, $MGSI = (W_g \times W_b^{-1}) - W_g \times 100$ (Nikolsky, 1963) and $D.I. = \sqrt[3]{W_g}$ (Dobriyal *et al.*, 1999), ovaries were weighed (W_g) to the nearest 0.001 g. Where W_b is total weight (g) and W_g is gonad weight (g). The length and age at first maturity were estimated for both sexes. Fecundity was measured in females captured in the reproductive period. In order to estimate fecundity, 3 subsamples with equal weight from the front, middle, and back of the ovary were sampled. The pieces were weighed and the eggs in them counted under a binocular microscope (Bagenal and Braum, 1978). The number of eggs in each female was calculated as the proportion of eggs in the sample to the weight of the whole ovary. To calculate absolute fecundity, ovaries recognized as four or five stage were used. The stage of gonad maturity was determined visually following the Nikolsky scale (1963). The relative fecundity (Fr) was expressed by dividing the absolute fecundity (Fa) by the fish body weight. The result was the number of eggs per gram of body weight (Bagenal, 1967). To determine the ovum diameter, the ovaries were preserved in Gilson fluid. The diameters of 30-60 ova of each female specimen were measured using a binocular microscope model M6C-10 which was fitted with an ocular micrometer.

To compare significant differences in the gonad indices among the samples taken on various months and various size samples, the analysis of variance and Tukey's test was applied ($p < 0.05$). The strength and significance of the relationship between the absolute fecundity (Fa) and selected individual features of the females included in the study (standard length, body weight, the gonad weight and the fish's age) were analyzed by determining Pearson's correlation coefficient r ($p < 0.05$) and regression equations (Thulasitha and Sivashanthini, 2013). The data were analyzed by the SPSS version 16.0 software package and Microsoft Excel 2010 software.

Results

In total, 316 specimens were collected during the sampling period, of which 179 (56.6%) were males and 137 (43.4%) were females. Mature males can be morphologically distinguished from the females by having large tubercles on the snout in a broad band below the nostril level, extending back under the eye and breaking up into a few tubercles on the operculum (Fig. 3). The female: male ratio was 1:1.31 (Table 1). The range of TL was from 59 mm to 196 mm for females and from 52 to 198 mm for males. Total mass was found to vary between 19.2 and 95.7 g in female and between 16.9 and 93.5 g in male (Table 1). This population of *C. macrostomum* had a narrow age range (1+ to 5+ years). The majority of the fish caught were 2+ (28.48%) and 3+ (34.49) years old, some were 1+ (14.24%) and 4+ (18.04) and only a

few of them were 5+ (4.75%) years old. Age at first maturity was more than one year about two years (+1) for males and

about three years (+2) for females. Size at first maturity was recorded 108 mm for females and 94 mm for males.



Figure 3: Lateral view of head of *Cyprinion macrostomum*; male, 142 mm SL, with breeding tubercles, above; female, 118 mm SL, below.

Table 1: Length and weight (mean \pm SD) in different age groups of males and females of *Cyprinion macrostomum* from Gamasiab River.

Age	n		SL [mm]		TL [mm]		W _b [g]	
	M	F	M	F	M	F	M	F
1+	26	19	89.5 \pm 3.7	88.1 \pm 2.9	108.2 \pm 2.1	108.0 \pm 4.5	18.5 \pm 3.2	18.8 \pm 3.5
2+	48	42	104.3 \pm 5.1	106.4 \pm 4.7	125.6 \pm 4.9	122.9 \pm 5.6	29.3 \pm 4.4	34.6 \pm 3.9
3+	61	48	122.7 \pm 6.3	119.8 \pm 5.0	146.8 \pm 4.6	144.8 \pm 5.5	38.1 \pm 5.0	42.9 \pm 5.1
4+	34	23	139.0 \pm 5.4	136.4 \pm 3.9	163.5 \pm 3.8	168.6 \pm 4.7	55.2 \pm 6.1	67.3 \pm 5.8
5+	10	5	151.2 \pm 3.0	160.1 \pm 3.4	184.3 \pm 2.4	190.1 \pm 3.3	78.6 \pm 3.9	89.8 \pm 3.3

SL = standard length; TL = total length; W_b = body weight; n = number of specimens; SD = standard deviation; M = male; F = female.

Following the seasonal cycle of the GSI (Fig. 4), three phases were identified in gonad activity: quiescence, maturation and the reproduction phase. The ovary began to develop between February and May after a quiescent period of 3 months (November – late January). The GSI of males and females during a year fluctuated and reached a peak in May, and reproduction during a year occurred from late May to mid-August, when water temperature reached 16-24°C. It thereafter decreases sharply in late

August. Then a secondary increase in ovaries development was observed from late August to mid-September, when some of the fish were ready to spawn. The GSI, MGSI and DI of both sexes followed the same pattern, but during the reproductive period (Figs. 4 and 5), the average values of males were significantly lower than those of females ($p < 0.05$). There were no significant differences between GSI and MGSI ($p > 0.05$).

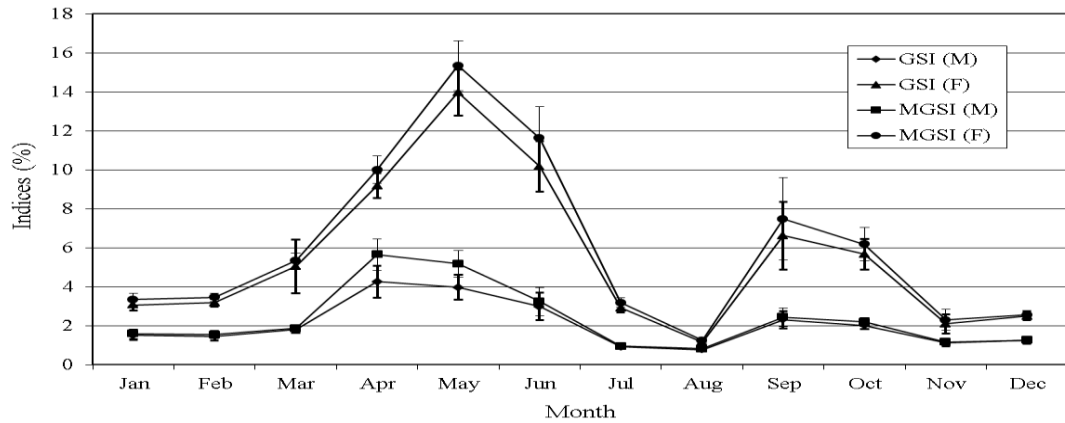


Figure 4: Variation of mean (\pm SE) gonadosomatic index (GSI) and modified gonadosomatic index (MGSI) of female (F) and male (M) for *Cyprinion macrostomum* from the Gamasiab River in different months.

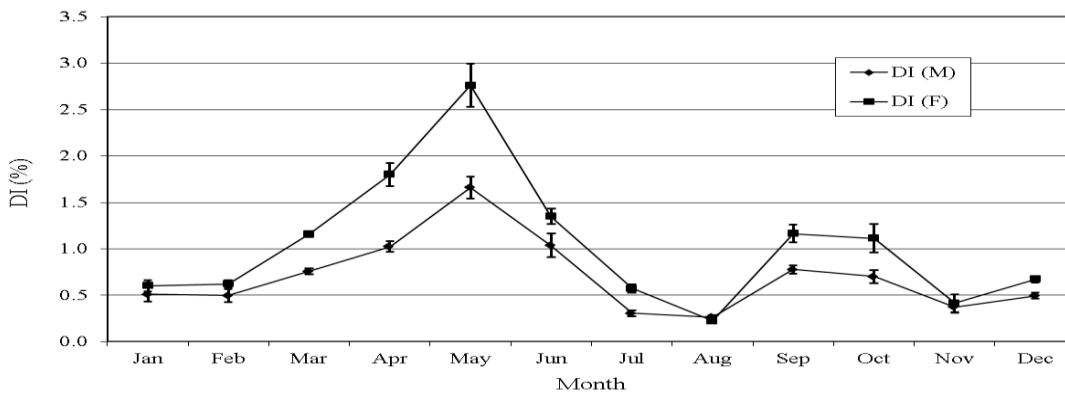


Figure 5: Variation of mean (\pm SE) Dobryial index (DI) of female (F) and male (M) for *Cyprinion macrostomum* from the Gamasiab River in different months.

Individual values of absolute fecundity estimated in 30 ovaries in the spawning season, varied in a wide range from 958 to 5629 eggs with an average of 3642.51 eggs ($SD \pm 1219.92$) (Table 2). Fecundity relative to total weight fluctuated from 38.1 to 67.9 eggs/g, with a mean value of 55.04 ± 14.12 (SD). The ovum diameters in the spawning season ranged from 0.2 to 1.7 mm with a mean of 0.9 mm

($SD \pm 0.43$). They were highest in May and lowest in August (Fig. 6). It was observed that the absolute fecundity accompanied the growth of weight of the female lotak. The absolute fecundity was significantly related to fish female body weight and also gonad weight ($p < 0.05$). The relationships of fecundity with body length and age were not found to be statistically significant ($p > 0.05$).

Table 2: Absolute (F_a) and relative (F_r) fecundity in particular ranges of body length (SL), body weight (W_b), and age of *Cyprinion macrostomum* females from Gamasiab River.

Parameter	n	F_a		F_r	
		Range	Mean \pm SD	Range	Mean \pm SD
SL [mm]	110.1–130	8	811–2106	1952 \pm 528	21–57 49 \pm 11
	130.1–150	17	1685–3911	3136 \pm 703	27–59 53 \pm 13
	≥ 150.1	5	3394–5629	4697 \pm 865	43–68 55 \pm 18

Table 2 continued:

	38.1–58	11	811–2436	2068 ± 587	21–58	50 ± 13
W_b [g]	58.1–78	14	1832–3970	3317 ± 811	28–59	54 ± 12
	≥78.1	5	3394–5629	4697 ± 865	43–68	55 ± 18
Age	3+	10	811–2365	1996 ± 545	21–59	50 ± 13
	4+	15	1714–3930	3206 ± 782	27–58	53 ± 12
	5+	5	3394–5629	4697 ± 865	43–68	55 ± 18

n = number of specimens; SD = standard deviation.

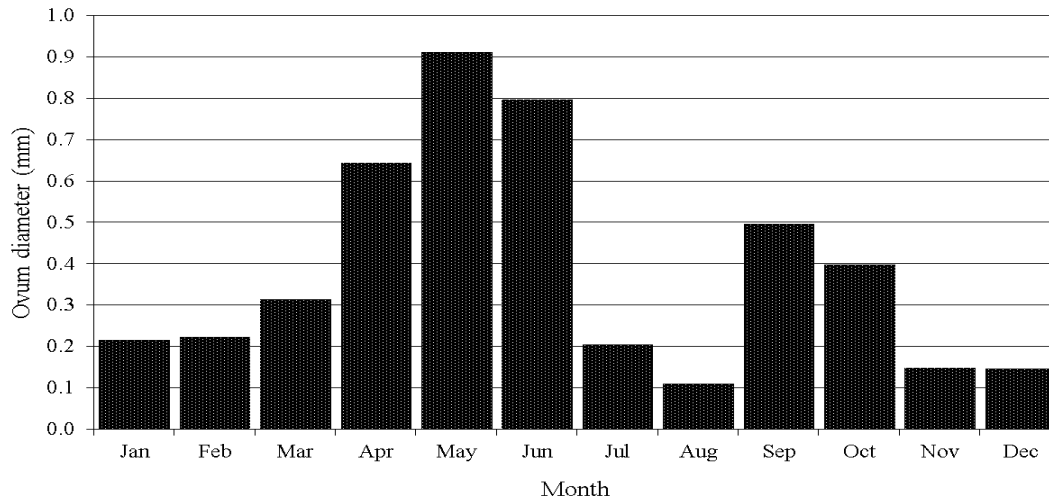


Figure 6: The mean oocyte diameter [mm] in the ovaries of *Cyprinion macrostomum* from the Gamasiab River in different months.

Discussion

The present study reports for the first time basic information on the reproduction biology of the "doctor fish" (or largemouth lotak), *C. macrostomum*, from Gamasiab River, in the Tigris River drainage. Previous reports on the body length of the "doctor fish" are similar to the sizes recorded in the present study (Coad, 2016). In the examined materials, the maximum SL was 162 mm, whereas Krupp (1985) stated that the fish reaches 193 mm in SL. The sex ratio was 1:1.31 (female: male). Sex determination in fish is a very flexible process with respect to evolutionary patterns observed among genera and families, and within individuals it is subject to modification by external factors (Robert and Nagahama, 2002).

These influences can affect the fate of both somatic and germ cells within the primordial gonad, and include the action of genetic, environmental (e.g. temperature), behavioral, and physiological factors (Robert and Nagahama, 2002). Exogenous sex steroids administered at the time of sex determination can strongly influence the course of sex differentiation in fish, suggesting that they play a critical role in assignment of gonad determination as well as subsequent differentiation (Robert and Nagahama, 2002). According to the obtained results, age at first maturity was more than one year about two years (+1) for males and about three years (+2) for females. Size at first maturity was recorded as 108 mm for females and 94 mm for males. Al-Rudainy (2008) also reported

maturity for *C. macrostomum* in Iraq at 2-3 years, but at 150 mm length and 50 g weight. Ünlü (2006) gives age at first maturity as 2 years in the Turkish Tigris River (upper Tigris) for *C. kais*. The difference between age at first maturity in the same genus may be caused by the difference in species, regions and habitat including different geographical distributions and environmental conditions, growth characteristics, also differences in exploitation and abundance for each stock (Avsar, 2001; Ismen, 2003; Yigin and Ismen, 2012; Mousavi-Sabet *et al.*, 2011; 2012a; 2012b; 2013; Faghani-Langroudi *et al.*, 2014).

The examined gonad indices in males and females fluctuated and reached a peak in May and reproduction latest till August, when water temperature was between 16-24 °C. In fish species which spawn in late spring and in summer (such as lotak, *C. macrostomum*), these indices remain low in winter and then rise sharply just before they spawn (Wootton, 1979). A rapid increase in the weight of ovaries takes place when the temperature rises and increasing amounts of food are consumed (Wootton, 1979). Al-Rudainy (2008) reported spawning season for *C. macrostomum* of May and June in Iraq on gravel beds in shallow water with fast currents. Also Ünlü (2006) observed spawning season for *C. kais* in the Turkish Tigris River over sand, stones and gravel in May-June. Spawning of *C. watsoni* (Day, 1872) takes place in Pakistan in Islamabad from mid to late March to mid-April (Shaikh and Jalali, 1989, 1991) and

near Islamabad (33.3°N, 73.0°E) in April and May (Shaikh and Hafeez, 1993). The spawning season of fish may vary with respect to the ecological characteristics of different water systems, such as water temperature and photoperiod, which have major effects on gonad maturation and spawning season (Bagenal and Braum, 1978; Wootton, 1990). Most reproduction studies on teleosts have focused on changes that occur during annual cycles in temperate species but, in tropical fish, this has been examined less frequently. The reproductive cycle of these species seems to be controlled by cues from the external environment. Relevant data about gonadal maturation, for example GSI and sexual hormones secretion, are contrasted (Guerrero *et al.*, 2009).

Estimated fecundity from 30 females caught in May and June, varied from 958 to 5629 eggs, whereas Coad (2016) reported up to 150 eggs from the "Ab Garm-e Ganow" (Genow hot spring) in *C. watsoni*. In general little is known about the reproductive biology of the genus *Cyprinion*, and detailed studies are unavailable. However, differences in number of eggs between species and/or populations can be attributed either to the effects of different environmental factors or to differences of species, since fecundity is affected by different environmental factors and may vary between different species (Nikolsky, 1963; Bagenal and Braum, 1978; Wootton, 1990). The oocyte size recorded in the present study was 0.2 to 1.7 mm in diameter. The maximum egg diameter reported for *C. macrostomum*

and *C. watsoni* were 1.4 mm and 1.2 mm, respectively (Coad, 2016). Also, Esmaeili and Gholamifard (2012) recorded oocyte diameter from 0.1 to 1.3 mm in *C. tenuiradius* from the Rudbal River, southern Iran.

According to the obtained results, it was concluded that this fish has a relatively prolonged active reproduction period, in the middle Tigris River drainage. Their reproduction in this region shows some differences from other available reports on the genus *Cyprinion*, which might be related to different species and environmental conditions. These results could be of interest for commercial exploitation, management and conservation programs of this species in the region.

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